6-WELL PLATE TRANSIENT COTRANSFECTION OF SHRNA PLASMID DNA INTO 293HEK CELLS

This protocol is adapted from Invitrogen's ViraPower™ Lentiviral Expression System by the Gene Expression Lab.
This protocol is for use with ViraPower™ Lentiviral Expression System. For additional technical inquiries, contact Technical Service at (800) 955-6288 *or www.invitrogen.com*

Recommendation:

- Follow the procedure below to transfect 293 cells. We recommend including a negative control (no DNA, no Lipofectamine™ 2000) in your experiment to help you evaluate your results. You will need 5 x 105 293 cells for each sample.

BEFORE STARTING THE EXPERIMENT

- Warm Opti-MEM I Medium without Serum AND with Serum to RT
- Thaw Plasmid DNA on ice
- Mix Lipofectamine 2000 well by vortexing

Transfection Procedure

1. The day before transfection, trypsinize and count the 293HEK cells and plate them so that they will be 80 to 90% confluent on the day of transfection
2. On the day of transfection, remove the culture medium from the 293HEK cells and replace with 2 mL of normal growth medium containing serum (Opti-MEM 1 Reduced serum Medium containing 2% serum)
3. Prepare DNA-Lipofectamine 2000 Complexes for each transfection sample
    a. Mix Lipofectamine 2000 gently before use, then dilute 10 µL in 250 µL of Opti-MEM I Medium withOUT serum – mix gently and incubate for 5 minutes at room temperature.

   b. While Lipofectamine mixture is incubating at RT, Dilute 500ng total plasmid DNA's (corresponding to the amount that sample should have) in 250 µL of Opti-MEM I Medium withOUT serum – mix gently.
   c. After the 5-minute incubation, combine the diluted DNA with the diluted Lipofectamine 2000 – Mix gently.
   d. Incubate for 20 minutes at RT to allow the DNA-Lipofectamine 2000 complexes to form.
4. Add the DNA-Lipofectamine 2000 complexes drop wise to each well, mix gently by rocking the plate back and forth. Incubate the cells O/N at 37˚C.
5. The next day (24 hours post transfection) – remove the medium containing the DNA-Lipofectamine 2000 complexes and replace with 2 mL complete media.
6. Approximately 48hours post –transfection, Remove growth medium from the cultured cells and wash 2x with 2 mL of PBS to rinse the cells on the bottom of the well. This will remove detached cells and residual growth medium.
   a. Take care to completely remove the rinse solution before adding Lysis Buffer.
7. Cell lysates may be rapidly prepared by manually scraping the cells from culture dishes in the presence of 250 µL of either: 1X Renilla Luciferase Assay Lysis Buffer; 1x Luciferase Assay Lysis Buffer; or Trypsin for FACs analysis of cells.
   a. Cells may be harvested immediately following the addition of Lysis Buffer or Trypsin.
8. If lysing cells for Renilla Luciferase (RL) Assay or Firefly Luciferase (FL) Assay, transfer the lysate into a tube for 1 or 2 freeze-thaw cycles to ensure complete lysis of cells.
   a. Generally, it is unnecessary to clear lysates of residual cell debris prior to performing the Renilla Luciferase Assay. However, if subsequent protein determinations are to be performed, we recommend clearing the lysate samples by centrifugation for 30 seconds in a refrigerated microcentrifuge.
   b. Transfer the cleared lysates to a fresh tube prior to reporter enzyme analyses.
   c. Store lysates at -70˚C until ready to perform cell analysis.
9. If Trypsinizing cells for FACs Analysis, after trypsinizing, remove cells from plate with 5% DMEM media and spin for 10' at max speed to pellet cells.
10. Then wash the cells 2X's with PBS, with 10' spins at max speed.
11. Finally, resuspend cells in 200 µL of PBS with 0.5% FBS and refrigerate at 4˚C until delivering to FACs core group for analysis.